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Localization of human chorionic gonadotropin beta subunit transcripts in ovarian cancer tissue

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Abstract: Recent studies demonstrated that besides placenta and malignant trophoblastic tumors, hCG and especially its β-subunit is secreted by a varieties of tumors of different origin. The aim of the present investigation was to determine the expression pattern of human chorionic gonadotropin gene in ovarian cancer tissue. The study included 8 patients with epithelial ovarian carcinoma. The expression and distribution of *hCG*β mRNA was assessed by *in situ* RT-PCR method. The semi-quantitative assessment was performed using computer image analysis. Transformation of the images into the pseudocolour scale showed a clear difference in fluorescence intensity among individual cancer cells. The intensity of ISRT-PCR products corresponding with expression level of *hCG*β demonstrated that its production by individual cancer cells is different. In all studied specimens of the ovarian carcinoma tissue, cancer cells characterized by the presence of active *hCG*β gene were found, whereas noncancerous tissue demonstrated lack of the gene expression. Thus, the study clearly shows that the expression of *hCG*β is the feature of ovarian cancer tissue.

Key words: *hCG*β - RT-PCR *in situ* - Pseudocolour images - Ovarian cancer

Introduction

Human chorionic gonadotropin (hCG) is a sialoglycoprotein hormone composed of two noncovalently linked subunits - α (hCGα) and β (hCGβ) [24]. Physiologically, hCG is produced by syncytiotrophoblastic cells of the placenta and secreted into blood, being present in urine of pregnant women and patients with trophoblastic diseases [3, 4]. The production of hCG, especially its β-subunit by patients with nontrophoblastic cancers has been reported by many authors and immunoreactive hCG/hCGβ has been detected in the blood of patients with a variety of tumors of different origin [3, 5, 8, 9, 11, 16, 20, 23, 25]. The presence of hCG in any of its forms is regarded as an *in vivo* phenotypic characteristic of human cancer cells [16].

The role of hCG in tumorogenesis is unknown but recent reports suggest that hCGβ can stimulate growth of cancer cells or inhibit the apoptosis and the elevated serum level of hCGβ correlates with higher aggressiveness of cancer and its resistance to the therapy [7, 15]. Intact hCG appears to be the main form of hCG immunoreactivity, whereas the expression of the free β subunit of hCGβ has been associated with metastatic phenotype of cancer cells, but the biological mechanisms behind this association remains unclear [8, 16, 23]. Although the serum immunoreactivity of hCG and hCGβ has been observed in patients with gynecological malignancies, there is almost no data of human chorionic gonadotropin expression in gynecological cancer tissue [12].

The aim of the present study was to analyze expression of human chorionic gonadotropin gene in epithelial ovarian cancer tissue.

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Materials and methods

Tissue samples. Surgical specimens of cancer tissue were obtained from 8 patients with epithelial ovarian carcinoma (median age 55 yrs, range 40-69 yrs), treated by surgery at the Department of Gynecologic Oncology, Poznań University of Medical Sciences in 2003. In all patients, histological confirmation of the cancer, including tumor grading, was obtained and the staging was performed according to the International Federation of Gynecology and Obstetric (FIGO). Histology groups were as follows: ovarian cancer of serous type (5 cases, tumor grading G1 - 1, G2 - 2, G3 - 2; FIGO IA - 1, IC - 1, IIC - 1, III - 2, IV - 1), ovarian cancer of mucinous type (1 case, tumor grading not determined, FIGO III), clear cell type (1 case, tumor

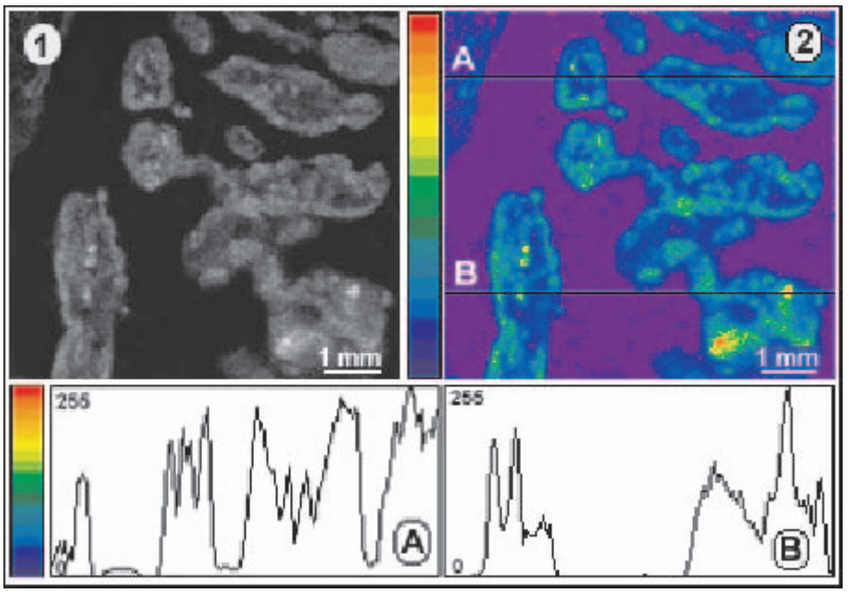

Fig. 1. The results of ISRT-PCR in ovarian cancer tissue. The cells containing *hCG* gene products are visible as bright spots. **Fig. 2**. The same image after transformation to pseudocolours. The cells containing *hCG* gene products are visible as yellow to red spots in cancer tissue. The weakly fluorescent green cells do not express *hCG*. **A**, **B**. The plots of the fluorescence intensity of the cells measured along lines A and B, respectively, shown in Fig. 2.

grading G2, FIGO III), and solid type (1 case, tumor grading G3, FIGO III).

The control samples obtained from the same patients consisted of surgically removed tissue which did not reveal any pathological changes after macroscopic and histopathological examinations: endometrium (2 samples) and the uterine cervix (1 sample) were collected. Two samples of placentas from term delivery served as a positive control.

The samples, collected at the operation were immediately fixed in 4% buffered formaldehyde, processed, embedded in paraffin and stored at 4°C. The patients were not treated by chemotherapy or radiotherapy prior to operation.

RT PCR *in situ*. In order to detect the *hCG*β mRNA, the paraffin sections were prepared according to the procedure described by Bagasra and coworkers [2, 24]. Reverse transcripton *in situ* was performed using Expand Reverse Transcriptase (Roche Molecular Biochemicals, Mannheim, Germany) with the primer specific for the *hCG*β gene (reverse primer: 5'-GAGAAGCCTTTATTGTG-3', complementary to nucleotides 51595-51611, PubMed, AC: NG000019). A 210 bp fragment of cDNA of the *hCG*β gene transcript was amplified by *in situ* PCR, using the following primers: sense 5'-GCAGGGGACGCACCAAGGA-3' (nucleotides 50499-50518 according to DNA sequence, PubMed, AC: NG000019) and antisense 5'- CACGCGGGTCATGGTGGG-3' (nucleotides: 51044-51296). The primers were designed to be complementary to the splice junction. Fluorescence labelled nucleotide Cy™3-dUTP (Amersham Biosciences, Little Chalfont Buckinghamshire, UK) was used in order to identify the amplified product. *hCG*β mRNA distribution was analysed using Carl Zeiss LSM 510 confocal microscope.

Computer generation of pseudocolours. The semi-quantitative analysis of *hCG*β expression was based on computer image analysis. Images generated in .lsm true colour file format from Zeiss LSM image Browser Version 3,1,0,99 for Carl Zeiss Laser Scanning System LSM 510 (Carl Zeiss GmbH) were exported to Microsoft® Windows® Bitmap File Format and saved. Using Adobe Photoshop® 5.5 CEEA (Adobe Systems Inc.1989-1999), colour mode of the images was changed to greyscale format (8 bit per channel; 256 grey levels) and stored as Microsoft Windows® 8 Bitmap File Format. Then, using the Scion Image® (Release beta 4.0.2) for Windows software (Scion Corporation© 2000), the greyscale was transformed into 32 colour table mode and stored as pseudocolour images.

Results

The results of the *in situ* RT-PCR (ISRT-PCR) study performed on paraffin sections of tumour tissue demonstrated the presence mRNA for beta subunit of human chorionic gonadotropin in all cases, both in placenta (data not shown) and in cancerous tissue. The distribution of *hCG*β mRNA in ovarian carcinoma was heterogeneous: not all cells of the studied tissue revealed the presence of the transcripts. The cells containing *hCG*β mRNA were usually organized in clusters characterized by uniform and homogenous cytoplasmic staining. Computer analysis and the use of pseudocolour scale showed a clear difference in fluorescence intensity among individual cancer cells. The intensity of ISRT-PCR products corresponding with expression level of *hCG*β demonstrated that its production by individual cancer cells is different (Figs. 1, 2). The fluorescence intensity measured after transformation into greyscale reached even maximal level for some cancer cells, whereas the other cells showed only weak or none fluorescence (Figs. A, B). In the control tissue from the same surgically removed material lacking the cancerous changes, the *hCG*β mRNA was not detected (data not shown).

Discussion

The present study was undertaken to determine the expression and distribution of *hCG*β mRNA in nontrophoblastic gynecological cancers. The study was based

on ISRT-PCR technique which allows for amplification of the signal, thus is more sensitive that *in situ* hybridization [2, 13, 18, 24]. The findings of our research indicate that mRNA of *hCG*β gene is present in ovarian carcinoma tissue. This is, to our knowledge, the first report, which directly revealed the production and distribution of hCGβ in ovarian tumors. At the same time we demonstrated that noncancerous tissue of the same women's genital tract did not demonstrate the expression of the gene. Thus, the present study shows that expression of *hCG*β is characteristic feature of the tumor tissue but its role in tumorogenesis is unknown.

The result of our study as well as other reports demonstrated that besides placenta and malignant trophoblastic diseases, a variety of tumors of different origin secrete hCG and especially its β-subunit [3, 11, 20]. Recently, hCGβ-related low molecular weight material has been demonstrated in the urine of pregnant women and patients with trophoblastic and nontrophoblastic tumors [14, 17, 21]. This material was termed hCGβ-CF because it retains the hCGβ core conformation determinant recognized by hCGβ core antiserum but lacks the carboxy-terminal portion of hCGβ [1]. Currently, β-CF is receiving attention as a potential tumor marker since elevated levels of β-CF were frequently found in the urine of patients with nontrophoblastic tumors even if immunoreactive hCGβ could not be detected in the serum [6, 19, 26]. The study performed by Higashida *et al.* demonstrated the production of immunoreactive human chorionic gonadotropin beta-subunit in case of one patient with ovarian malignant mixed mesodermal tumor [10].

Our study based on ISRT-PCR technique showed the distribution of cancer cells expressing the *hCG*β in ovarian tumors. The positively stained cells were present in all studied cancer tissue samples, however, in some cases only single cells expressing *hCG*β were observed. The tissue lacking the cancerous changes did not show the presence of *hCG*β mRNA. Computer analysis and use of pseudocolour scale showed the difference in fluorescence intensity among individual cancer cells of the analysed tissue. The intensity of ISRT-PCR product fluorescence corresponding with the expression level of *hCG*β showed that its production by individual cells is different. Further investigations should allow to determine the type of cancer cells expressing *hCG*β and to find out whether and when the cells posses the ability to produce hCGβ in preneoplastic lesions.

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